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Harvey Sarcoma Virus: A Second Murine Type C Sarcoma Virus with Rat Genetic Information

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The nucleic acid sequences found in the Harvey strain of murine sarcoma virus have been analyzed by RNA·[^{3}H]DNA and [^{3}H]RNA·DNA hybridization techniques. The Harvey strain of murine sarcoma virus has been found to possess at least two sets of nucleic acid sequences. One set of sequences is contained in the Moloney strain of mouse type-C virus, and the other set is contained in DNA transcripts synthesized in endogenous reactions containing rat type-C virus(es). The nucleic acid sequences that are detected in the Harvey sarcoma virus with the DNA probes synthesized from the rat type-C virus(es) are related to the rat sequences detected in the Kirsten strain of murine sarcoma virus. The results support the model that both Kirsten and Harvey sarcoma viruses arose through a process of recombination or reassortment between mouse type-C viruses and sequences in rat cells and suggest that the information for transformation of fibroblasts may be contained in the rat type-C or cellular genome.

RNA-containing type-C viruses have been classified into two broad groups of viruses based on their biological properties. One type, the "leukemia viruses," are able to productively infect fibroblast cell cultures without morphologically altering the cells; the other type, "sarcoma viruses," are transforming viruses and produce morphological transformation of fibroblasts in cell culture. The helper type-C viruses or leukemia viruses have been isolated from many species. Isolates of sarcoma viruses have been more limited but such transforming viruses have been isolated from chickens (22), mice (7, 19), rats (9, 14), cats (8, 25), and recently from a woolly monkey (27). The mode of isolation of sarcoma virus has varied from species to species. For example, in the case of sarcoma virus isolates from cats (8, 25) and the woolly monkey (27), transforming virus was isolated directly from extracts of spontaneously occurring tumors.

In mice, several strains of sarcoma virus have been isolated. One strain, isolated from a spontaneously occurring osteosarcoma (7), the FBJ strain, only recently has been studied in cell culture (16) and little is known about its genome. Three other isolates of murine sarcoma virus have been obtained by animal passage of nontransforming helper type-C virus and the subsequent isolation from the stock of two viruses, the original helper, and a transforming or sarcoma virus which can produce nonlymphoid soft tissue tumors. Examples of such experiments are the isolation of Moloney sarcoma virus in mice (19), the isolation of the Kirsten sarcoma virus by passage of Kirsten murine erythroblastosis virus in rats (14), and the isolation of the Harvey sarcoma virus by passage of the Moloney helper type-C virus in rats (9).

Earlier studies (3, 24) of the Kirsten and Moloney sarcoma viruses by hybridization of the nucleic acid sequences showed that the RNA nucleic acid sequences present in the Moloney sarcoma virus and the Kirsten sarcoma virus differed. Because of the low degree of homology between our DNA transcripts from mouse and rat type-C viruses, it was possible to insure that the RNA sequences measured in the heterologous sarcoma virus-transformed nonproducer cell were of the sarcoma virus itself and not due to the endogenous type-C virus(es) of that cell. The Kirsten strain of sarcoma virus, but not the Moloney strain, was found to contain sequences homologous to those found in a rat type-C virus(es). In addition, both the Moloney and Kirsten sarcoma viruses were found to contain sequences homologous to the mouse type-C virus which was present in the helper virus used in the original isolation of the respective sarcoma viruses.

The present studies were designed to explore whether the Kirsten sarcoma virus was a unique virus or whether other strains of murine sarcoma virus might also contain rat type-C viral sequences. We have examined the nucleic acid sequences of the Harvey strain of murine sarcoma virus. Since this virus was isolated by

passage of Moloney type-C helper virus in rats, nonproducer cells transformed by the Harvey sarcoma virus in the absence of replicating type-C virus were derived; cellular RNA from such nonproducer cells was then analyzed for the types of nucleic acid sequences. The results indicate that the Harvey strain is similar to the Kirsten strain of sarcoma virus in that it may also be a recombinant between a murine type-C virus and nucleic acid sequences found in rat cells.

MATERIALS AND METHODS

Cells. All cells were grown in Dulbecco's modification of Eagle medium with 10% calf serum (Colorado Serum Co.). The source of the NIH 3T3 and normal rat kidney (NRK) cells has been previously described (1, 11). The source of the nonproducer rat cells transformed by either the HT-1 strain or S^+L^- strain of Moloney sarcoma virus and the nonproducer NIH mouse or NRK rat cells transformed by Kirsten sarcoma virus has also been previously reported (2, 3, 10). The nonproducer NIH or NRK cells transformed by the Harvey sarcoma virus were obtained by serial dilution of filtered sarcoma virus stocks, described below, and isolation of transformed cells in either soft agar or Falcon microtest II plates as previously described. The NRK cell spontaneously producing endogenous rat type-C virus(es) was the gift of George Todaro and Raoul Benveniste, National Cancer Institute (4).

The following abbreviations are used throughout the manuscript. The Kirsten sarcoma virus-transformed cells are referred to as either Ki-NIH or Ki-NRK. The Harvey sarcoma virus-transformed cells are referred to as either Ha-NIH or Ha-NRK cells. The Moloney sarcoma virus-transformed cells are referred to by the strain of the Moloney sarcoma virus used as either the S^+L^--NRK or Mo-NRK in the case of the HT-1 strain of the Moloney sarcoma virus (10).

Viruses. The source and growth of the Kirsten strain of murine leukemia virus (Ki-MuLV) and Kirsten sarcoma virus (Ki-SV) has been described (3). The source of Moloney leukemia virus (Mo-MuLV) has also been reported (2). The Gibbon ape type-C virus (GaLV) (13) was obtained from a human cell line producing the Gibbon ape type-C virus (4). A pseudotype of the Kirsten sarcoma virus with the Gibbon ape leukemia virus Ki-SV (GaLV) was obtained by infecting Ki-NRK nonproducer rat cells with the Gibbon ape type-C virus and preparing a virus preparation from this supernatant of such cells. The Harvey sarcoma virus was obtained from Wallace Rowe and Janet Hartley, National Institute of Allergy and Infectious Diseases, Bethesda, Md., as a stock of virus containing both the Moloney leukemia virus and the Harvey sarcoma virus. This virus mixture was derived from a pool of Harvey sarcoma virus from rat plasma subsequently transmitted to NIH mouse cells. The virus stock passaged through NIH mouse cells was used by us to isolate the NIH mouse nonproducer cells transformed by the Harvey sarcoma virus as described above. To obtain the rat cell nonproducer cells transformed by the Harvey sarcoma virus, the Ha-NIH nonproducer cells were rescued with the Moloney type-C virus as previously described (1, 25) and nonproducer Ha-NRK foci isolated as described above. Neither the mouse cell nor rat cell nonproducer released any particles as judged by electron microscopy, uridine labeling, or assay of culture supernatants for virus reverse transcriptase. These Harvey nonproducers are similar to those reported by Levy (17).

Synthesis of virus-specific [^{3}H]DNA. The endogenous reverse transcriptase reaction from sucrose density gradient-banded viruses was used as the source of all products as previously described (3); actinomycin D at 40 to 60 μg/ml was included in all reactions. For the tritium-labeled DNA products, [^{3}H]dCTP was used as label (NEN, specific activity, 20 Ci/mmol). With the [^{3}H]DNA probe synthesized from the rat type-C virus-containing preparations, the product was purified to insure that the [^{3}H]DNA synthesized represented sequences contained in 60 to 70*S* viral RNA. To do this, the [^{3}H]DNA product synthesized in an endogenous reaction for 1 to 4 h was deproteinized with phenol and chloroform and precipitated with ethanol with 500 μg of carrier yeast RNA per ml. The [^{3}H]DNA bound to 60 to 70*S* RNA was isolated on sucrose gradients as previously described by the simultaneous detection assay of Schlom and Spiegelman (23). All [^{3}H]DNAs were then treated with 0.4 M KOH as described to remove RNA, dialyzed, and stored at −20 C before use.

The unlabeled DNA transcripts of the Moloney luekemia virus, the Kirsten leukemia virus, or the rat type-C virus were synthesized in parallel reactions containing unlabeled dCTP in place of the [^{3}H]dCTP at a molarity of dCTP equal to that used in the reactions containing the [^{3}H]dCTP, namely, 5×10^{-6} M. The amount of unlabeled DNA synthesized was then estimated from the yield in a parallel reaction containing [^{3}H]dCTP. This calculation was based on the specific activity of the [^{3}H]dCTP, the assumption that all four bases are incorporated equally into the DNA transcript, and the determination by alkaline sucrose sedimentation of the molecular weight of various labeled transcripts which was found to be between 150,000 and 300,000. Details of the alkaline sucrose procedure have been previously given (21).

Isolation of [^{3}H]60 to 70S RNA. To obtain [^{3}H]60 to 70*S* RNA from various viral cultures, a modification of the procedure of Nieman (20) was employed: [^{3}H]uridine (42 mCi/mmol), [^{3}H]cytidine (26 mCi/mmol), and [^{3}H]adenosine (32 mCi/mmol) were added to culture media at 10 to 20 μCi each per ml. Cultures were incubated for 8 to 12 h at 37 C, and the media was removed and saved at 4 C. Fresh media containing new isotope as above was then added, and the cells were incubated for an additional 8 h. The two harvests of labeled virus were then processed as follows. Saturated ammonium sulfate, buffered in 0.05 M Tris-hydrochloride, pH 7.5, and 0.01 M EDTA, was added to 50% saturation and the precipitate was harvested at 4 C by centrifugation at 10,000 × *g* for 15 min. The precipitate was resuspended in

approximately 30 ml per original 600 ml of culture fluid in buffer containing 0.05 M Tris-hydrochloride, pH 7.8, 0.1 M sodium chloride, and 10^{-3} M EDTA (TNE). The resuspended virus was layered on top of a discontinuous sucrose gradient in an SW 27 rotor tube containing the following sucrose solutions in the same TNE buffer: 4 ml of 60% sucrose, 12 ml of 30% sucrose, and 10 ml of 20% sucrose. The gradients were spun for 16 to 20 h at 25,000 rpm, 4 C, and the virus band on the 60% cushion was aspirated. The virus was then concentrated and removed from the sucrose by dilution in TNE buffer minus sucrose and by centrifugation at 100,000 × *g* at 4 C for 2 h. The pelleted virus was then suspended in TNE buffer containing 1% SDS and layered on top of a 15 to 30% glycerol gradient containing TNE and 0.1% SDS. It was centrifuged for 2 h in an SW 41 rotor at 35,000 rpm at 20 C. The gradient was punctured and collected from below into a 0.4-ml fraction and the [^{3}H]RNA peak centrifuging at 60 to 70*S* was pooled, dialyzed against TNE, and lyophilized. It was stored at −20 C and lyophilized until use. The specific activities of the various RNA preparations are given in the appropriate legends and varied from 4×10^4 counts per min per μg to 3×10^5 to 4×10^5 counts per min per μg.

Hybridization. Hybridization conditions and analysis of the [^{3}H]DNA·RNA hybridization reactions by s1 nuclease have been previously described (3, 24). Total cellular RNA was extracted also as previously described. S1 nuclease was used also to analyze the [^{3}H]RNA·DNA hybridization reactions; approximately two-fold more s1 enzyme was required to degrade the [^{3}H]RNA than the [^{3}H]DNA in the absence of hybridization. Exact conditions for each hybridization are given in the legends to appropriate tables and figures.

RESULTS

Relatedness of Ha-SV and Ki-SV. Our earlier studies had shown that we could distinguish, by RNA·[^{3}H]DNA hybridization, the nucleic acid sequences of the Moloney strain of murine sarcoma virus from the nucleic acid sequences of the Kirsten strain of murine sarcoma virus. Since the Harvey strain of murine sarcoma virus was derived independently from passage of Moloney murine leukemia virus in rats, we examined the relatedness of the Kirsten, Moloney, and Harvey sarcoma viruses by RNA·[^{3}H]DNA hybridization. To examine the sarcoma virus RNA in the absence of replicating leukemia virus, total cellular RNA from NIH 3T3 cells nonproductively transformed by each of these sarcoma viruses was prepared. A [^{3}H]DNA product was made from a virus preparation containing the Kirsten sarcoma virus and a heterologous helper virus, the Gibbon ape type-C virus. This [^{3}H]DNA probe, potentially containing sequences from both the Kirsten sarcoma virus and the Gibbon ape leukemia virus, was then hybridized to each of these cellular RNAs. The results are shown in Fig. 1. RNA from a nonproducer NIH mouse cell transformed by the Kirsten sarcoma virus hybridized well to this [^{3}H]DNA transcript, with almost 80% of the input counts hybridized at saturating levels of RNA. RNA from an NIH mouse cell transformed and nonproductively infected by the Harvey sarcoma virus hybridized at saturation to approximately 55% of the input [^{3}H]DNA counts. In contrast, the RNA from nonproducer NIH mouse cells transformed by either the S^+L^- strain or HT-1 strain of Moloney sarcoma virus hybridized to only 10% of the input [^{3}H]DNA product. As a further control, the Moloney sarcoma virus-transformed

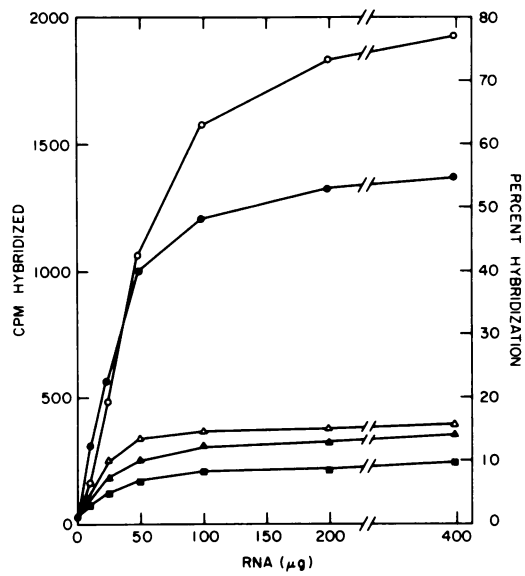

FIG. 1. *Hybridization of [^{3}H]DNA product from Ki-SV (GaLV) to various cellular RNAs. Each hybridization reaction mixture of 0.20 ml was incubated at 66 C for 24 h in 0.02 M Tris-hydrochloride, pH 7.2, 0.40 M sodium chloride, 10^{-4} M EDTA, 0.1% SDS, 20 μg of yeast RNA, approximately 2,500 trichloroacetic acid counts/min of [^{3}H]DNA, and the various cellular RNAs as detailed below. Hybridization was measured with the use of s1 nuclease as previously described. Each RNA preparation was tested for zero time inhibition of the s1 nuclease at the highest input level of RNA and was found not to inhibit the nuclease (less than 1% of the input counts). A 25-μg amount of s1 nuclease was used per assay point. Symbols: ○, RNA from Ki-NIH cells; ●, RNA from Ha-NIH cells; □, RNA from S^+L^- or Mo-NIH cells; ■, RNA from NIH cells producing the HT-1 strain of Moloney sarcoma virus and Moloney leukemia virus or the S^+L^- strain of Moloney sarcoma virus and Moloney luekemia virus; ▲, RNA from NIH cells producing Kirsten murine type-C virus; △, RNA from a human cell line A204 producing the Gibbon ape type-C virus.*

nonproducer cells were superinfected with Moloney leukemia virus to rescue infectious sarcoma virus. The cellular RNA from these cells producing either strain of Moloney sarcoma virus and Mo-MuLV still hybridized to only 10 to 13% of the input [^{3}H]DNA counts. To assess the role of sequences in the [^{3}H]DNA probe homologous to the Gibbon ape leukemia virus, RNA from a human cell producing the Gibbon ape leukemia virus was hybridized to the [^{3}H]DNA transcript. At saturation, only 15% of the input counts hybridized. A similar final level of hybridization was also achieved with RNA from NIH mouse cells productively infected with the Kirsten murine leukemia virus. The results indicate that the RNA from mouse cells transformed by the Harvey sarcoma virus is more closely related to sequences contained in the Kirsten sarcoma virus than to sequences in the Moloney sarcoma virus, Moloney murine leukemia virus, or Kirsten murine leukemia virus. The results further indicate that the majority of the Ki-SV and Ha-SV related sequences which hybridize to the [^{3}H]DNA probe containing both the Gibbon type-C virus and the Kirsten sarcoma virus are not contained in the Gibbon ape leukemia virus sequences in this probe. Thus, the results suggest that the nucleic acid sequences in the Kirsten strain of sarcoma virus and the Harvey strain of sarcoma virus are closely related.

Rat sequences in Ha-SV. To explore quantitatively the related sequences between the Harvey sarcoma virus and the Kirsten sarcoma virus, a [^{3}H]DNA probe was synthesized from a reverse transcriptase endogenous reaction containing rat type-C virus(es) spontaneously produced from NRK cells. The results are shown in Fig. 2. RNA from rat cells producing these type-C virus(es) hybridized to almost 60% of the input [^{3}H]DNA counts. In contrast, the RNA from mouse cells infected with either Moloney luekemia virus, either strain of Moloney sarcoma virus, Moloney sarcoma and leukemia virus, or Kirsten leukemia virus hybridized to less than 3% of the input [^{3}H]DNA counts. As previously reported, the RNA from similar NIH nonproducer mouse cells transformed by the Kirsten sarcoma virus hybridized readily to the [^{3}H]DNA probe made from the endogenous reaction containing rat type-C virus(es); in addition RNA from NIH nonproducer mouse cells transformed by the Harvey sarcoma virus also readily hybridized to this [^{3}H]DNA probe. The results indicate that both the Kirsten strain of sarcoma virus and the Harvey strain of sarcoma virus contain as part of their genomes sequences that hybridize to the [^{3}H]DNA transcript prepared from an endogenous reaction containing rat type-C virus(es). To ascertain if the rat sequences detected in the reactions with Kirsten or Harvey sarcoma virus RNA were similar or different, saturating levels of each of the two RNAs were mixed and hybridized to the [^{3}H]DNA transcript prepared from the rat type-C virus(es). In results not shown, such experiments indicated that all of the sequences detected came from overlapping regions.

[^{3}H]RNA·DNA hybridization. Since the [^{3}H]DNA single-stranded probes used might not be representative of the entire viral genomes, a series of unlabeled single-stranded DNA transcripts of Moloney leukemia virus, rat type-C virus, and Kirsten type-C virus were synthesized. Tritiated 60 to 70*S* RNA was also prepared from a series of type-C viruses. The unlabeled DNA transcript prepared from the endogenous reaction containing the rat type-C virus(es) was hybridized to DNA excess to this series of [^{3}H]60 to 70*S* RNAs, and the results are shown in Fig. 3. In Fig. 3A it can be seen that the unlabeled DNA transcript from the rat type-C virus(es) hybridized well (34%) to the [^{3}H]RNA from the virion preparation containing both the Moloney leukemia virus and the Harvey sarcoma virus; less than 2% of the Moloney [^{3}H]60 to 70*S* RNA hybridized to this same DNA transcript. Control studies showed, in data not shown, that an unlabeled DNA

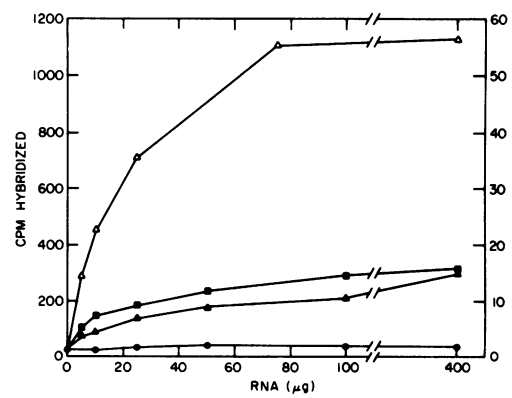

FIG. 2. *Hybridization of [^{3}H]DNA from rat type-C virus with various cellular RNAs. The reaction conditions are as in the Fig. 1 legend. Each reaction contained approximately 1,800 trichloroacetic acid counts/min of [^{3}H]DNA. Symbols: △, RNA from NRK cells producing rat type-C virus; ■, RNA from Ha-NIH cells; ▲, RNA from Ki-NIH cells; ●, RNA from NIH cells producing Mo-MuLV; ○, RNA from NIH cells producing the HT-1 strain of S$^+$L$^-$ strain of Moloney sarcoma virus and the Moloney leukemia virus.*

product of Moloney luekemia virus hybridized to greater than 95% of the [^{3}H]60 to 70*S* RNA counts of the Moloney leukemia virus RNA itself. In Fig. 3B, the same unlabeled rat virus DNA probe hybridized well to the high molecular weight RNA preparation from the Kirsten strain of sarcoma virus and Kirsten strain of murine leukemia virus mixture, but not to the [^{3}H]60 to 70*S* RNA of the Kirsten murine leukemia virus alone. In data not shown, control studies showed that the unlabeled DNA transcript of the Kirsten leukemia virus hybridized to greater than 90% of the Kirsten [^{3}H]60 to 70*S* RNA itself. In Fig. 3C the same rat DNA probe was hybridized to [^{3}H]60 to 70*S* RNA made from the rat type-C virus; at saturation approximately 75% of the [^{3}H]RNA counts hybridized. The unlabeled Moloney DNA transcript did not appreciably hybridize (<2%) to this same [^{3}H]RaLV RNA. From both the RNA versus [^{3}H]DNA and the [^{3}H]RNA versus unlabeled DNA hybridization experiments, the results indicate that the Kirsten and Harvey strains of sarcoma virus both contain sequences found in DNA transcripts of rat type-C virus(es). The presumed rat type-C viral sequences are not found in the Moloney strain of murine type-C virus, the Kirsten strain of murine type-C virus, or either the S^+L^- strain or the HT-1 strain of Moloney murine sarcoma virus.

Mouse type-C sequences in Ha-SV. Earlier studies had shown that both the Moloney and Kirsten strains of murine sarcoma virus contain sequences homologous to murine type-C virus. To examine the Harvey sarcoma virus for sequences other than those found in the rat type-C virus, RNA from rat cells nonproductively transformed by the Harvey sarcoma virus was hybridized to a [^{3}H]DNA transcript made from the Moloney leukemia virus. The results are shown in Fig. 4. RNA from these cells hybridized to 28% of the input Moloney [^{3}H]DNA counts. This 28% level of hybridization was less at its final extent than that found with either the RNA from the rat cells transformed by the S^+L^- strain of Moloney sarcoma virus or RNA from rat cells transformed by the HT-1 strain of Moloney sarcoma virus. The RNA from rat cells productively infected with Moloney leukemia virus hybridized well to this same Moloney DNA transcript with 80% of counts resistant to s1 nuclease at the extent of hybridization; conversely, rat cells productively making the endogenous virus(es) of NRK hybridized to less than 3% of the input counts. A similar result was observed using three separate strains of Harvey sarcoma virus isolated from the initial mixture as compared to either of the Moloney sarcoma virus-transformed rat cells.

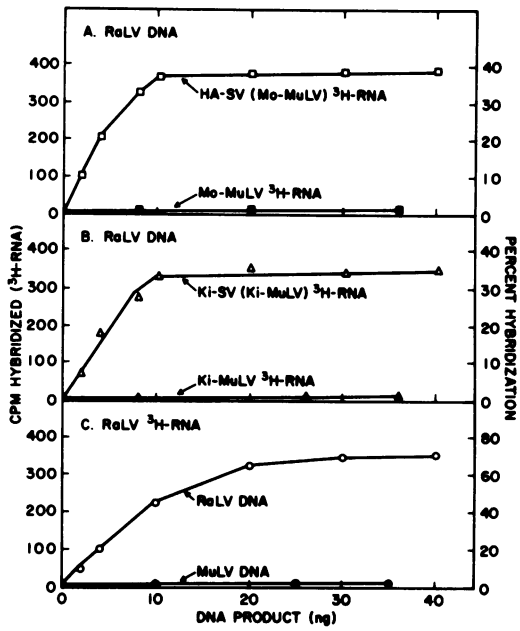

FIG. 3. *Hybridization of various [^{3}H]60 to 70S RNAs to unlabeled DNA transcripts. (A) Each reaction mixture was hybridized in 0.10 ml of reaction mixture at 66 C for 24 h under conditions described in the legend to Fig. 1. S1 nuclease was used to degrade the unhybridized [^{3}H]RNA under the same conditions as used in s1 nuclease reactions to degrade the single-stranded [^{3}H]DNA exept that 50 μg of s1 was used per assay point. Thus the formation of the [^{3}H]RNA·DNA hybrid was monitored by s1 nuclease as was the formation of the [^{3}H]DNA·RNA hybrids in the legend to Fig. 1. Symbols: □, [^{3}H]RNA from a mixture of Harvey sarcoma virus and Moloney leukemia virus growing in NIH 3T3 cells; ■, [^{3}H]RNA from Moloney leukemia virus growing in NIH 3T3 cells. The specific activity of the input [^{3}H]RNAs was approximately 2 × 10^6 counts/min per μg and each reaction contained approximately 800 counts/minute of [^{3}H]RNA (~4 ng). (B) Details are as in 4A except the [^{3}H]RNA (△) was from Ki-MuLV and Ki-SV growing in NIH 3T3 cells; or (▲) Ki-MuLV [^{3}H]RNA from Ki-MuLV growing in NIH 3T3 cells. Specific activity of the RNA preparation was approximately 3 × 10^5 counts/min per μg and each reaction mixture contained approximately 700–800 counts per minute of [^{3}H]RNA (~4 ng). (C) Conditions are as in the legend to 4A. The [^{3}H]RNA used in this reaction was from the rat type-C virus and has a specific activity of 6 × 10^4 counts/min per μg and each reaction mixture contained approximately 500 trichloroacetic acid counts/min of [^{3}H]RNA (~10 ng), and it was hybridized to either the unlabeled DNA product from the rat type-C virus (○); or the DNA from either Ki-MuLV or Moloney MuLV (■).*

Since the final level of hybridization achieved

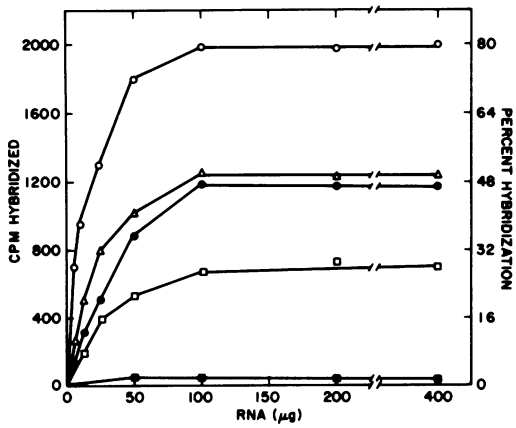

FIG. 4. *Hybridization of [^{3}H]DNA from Moloney leukemia virus to various cellular RNAs. Reaction conditions are as in the legend to Fig. 1. Each reaction contained approximately 2,500 trichloroacetic acid counts/min of [^{3}H]DNA. Symbols: ○, RNA from NRK cells producing Moloney leukemia virus; △, RNA from S$^+$L$^-$ NRK cells; ●, RNA from Mo-NRK cells (HT-1 strain of Moloney sarcoma virus); □, RNA from Ha-NRK cells; ■, RNA from NRK cells producing rat type-C virus.*

with the RNA from cells productively infected with Moloney leukemia virus was higher than that with cellular RNA from cells nonproductively infected with the various strains of Moloney and Harvey sarcoma virus, a series of mixing experiments with the various RNAs were performed to see if we could detect different mouse type-C viral information in the various strains of Moloney and Harvey sarcoma virus. The results are shown in Table 1. In experiment 1, Table 1, increasing levels of RNA from Harvey sarcoma virus-transformed rat cells were added to saturating levels of cellular RNA from rat cells transformed by the HT-1 strain of Moloney sarcoma virus. Addition of the Harvey cellular RNA did not increase the final extent of hybridization achieved with the cellular RNA from the HT-1 Moloney sarcoma virus transformed rat cells. In a reciprocal experiment, addition of increasing amounts of the cellular RNA from the HT-1 Moloney sarcoma virus-transformed rat cell to saturating levels of the Harvey sarcoma virus RNA did yield a final level of hybridization similar to that of the HT-1 strain of Moloney sarcoma virus by itself. These results indicate that the cellular RNA from the rat cells transformed by the HT-1 strain of Moloney sarcoma virus contains sequences which are not contained in the Harvey sarcoma virus cellular RNA, and that the cellular RNA from the Harvey sarcoma virus-transformed rat cell is missing some sequences found in the cellular RNA of the Moloney sarcoma virus-transformed rat cell.

In experiment 2, Table 1, the cellular RNA from rat cells transformed by the S$^+$L$^-$ strain of Moloney sarcoma virus was mixed with either cellular RNA from the HT-1 Moloney sarcoma virus-transformed rat cell or the Harvey sarcoma virus-transformed rat cell. In neither case did the final level of hybridization exceed that observed with the S$^+$L$^-$ strain of Moloney

TABLE 1. *Hybridization with cellular RNA from Moloney and Harvey sarcoma virus nonproducer rat cells*

Cellular RNA added (μg)			Hybridized (counts/min)
S$^+$L-NRK	MO-NRK	HA-NRK	
Expt 1			
—	50	—	805
—	100	—	1,189
—	200	—	1,207
—	400	—	1,195
—	—	50	590
—	—	100	769
—	—	200	750
—	—	400	783
—	200	50	1,226
—	200	100	1,051
—	200	200	1,103
—	200	400	1,255
—	25	200	913
—	50	200	1,166
—	100	200	1,280
—	200	200	1,193
Expt 2			
200	—	—	1,350
200	—	—	1,385
200	25	—	1,275
200	50	—	1,310
200	100	—	1,433
200	200	—	1,390
200	—	50	1,378
200	—	100	1,110
200	—	200	1,268
200	—	400	1,389 (1,980[a])

[a] Hybridization was with the [^{3}H]DNA transcript from the Moloney type-C virus grown in NIH 3T3 cells. Each reaction mixture contained approximately 2,500 trichloroacetic acid counts/min. The counts per minute hybridized in parenthesis indicate the number of counts per minute hybridized with RNA from rat cells producing Moloney leukemia virus. Only 25 counts/min were retained on Millipore filters in the absence of any RNA added after degradation of the [^{3}H]DNA transcript with s1 nuclease, and zero time additions of any of the mixtures of RNAs indicated in the table at the highest input levels of RNA failed to inhibit the s1 nuclease (less than 1%).

sarcoma virus alone. The results indicate that the mouse type-C viral sequences contained in total cellular RNA from rat cells transformed by the S^+L^- strain of Moloney sarcoma virus contains all of the sequences found in the cellular RNA from the rat cells transformed by either the Harvey strain of sarcoma virus or the HT-1 strain of Moloney sarcoma virus and that the cellular RNA from both of the latter two transformed cells lacks sequences contained in the S^+L^- cellular RNA.

DISCUSSION

Our previous findings in studies on the Kirsten strain of murine sarcoma virus indicated that the Kirsten sarcoma virus genome contained at least two distinguishable sets of nucleic acid sequences (24). One set was homologous to nucleic acid sequences found in the Kirsten strain of mouse type-C virus. The other set was found to be homologous to a [^{3}H]DNA transcript which was synthesized in an endogenous reverse transcriptase reaction containing rat type-C virus(es). Since Kirsten sarcoma virus was formed by passage of Kirsten mouse type-C virus in rats (14), we proposed that Kirsten sarcoma virus might represent a recombinant between Ki-MuLV and sequences found in rat cells.

The Harvey strain of murine sarcoma virus was isolated independently by passage of Moloney mouse type-C virus in rats (9). Thus, we undertook similar hybridization experiments to ascertain if the Harvey sarcoma virus also was a recombinant between a mouse type-C virus and nucleic acid sequences found in rat cells. Our results indicate that the Harvey strain of murine sarcoma virus also contains at least two distinguishable sets of nucleic acid sequences. One set is homologous to nucleic acid sequences present in the Moloney strain of murine type-C virus; the other set is present in sequences found in endogenous virus(es) released from NRK rat cells. Thus, in both independent cases, the formation of a sarcoma virus was accompanied by a recombinant event. In mixing experiments, the rat type-C viral nucleic acid sequences were found to be similar for both the Kirsten and Harvey sarcoma viruses. However, we do not know whether the rat sequences that we detect are responsible for the transforming activity of each of these sarcoma viruses.

In addition to the evidence that the Kirsten and Harvey strains of sarcoma virus have gained new sequences not present in the mouse type-C viruses which went into their formation, the present studies and earlier studies have demonstrated that each of these two sarcoma viruses also contain mouse type-C viral sequences. However, in each case not all of the sequences present in the initial murine mouse type-C virus are detected in the total cellular RNA from nonproducer cells transformed by either the Kirsten sarcoma virus or Harvey sarcoma virus. It is possible that the reason these sequences are missing is that they are present in the DNA of such nonproducer cells but are not transcribed in such nonproducer cells. Another possibility is that the sequences have been deleted either during or after the process of reassortment and/or recombination and are now missing from the sarcoma virus genomes. Although the evidence is not yet definitive, the latter interpretation would be supported by the observations of Maisel and her co-workers (18), which have shown that both Kirsten and Harvey strains of sarcoma virus have a smaller genome than is present in a complete murine type-C helper virus. This would be consistent with the idea that part of the initial genome had been deleted during the process of integration, reassortment or recombination. In addition, preliminary studies of the DNA in such sarcoma virus-transformed cells would also suggest that only part of the Moloney or Kirsten type-C genomes are present in the DNA of the sarcoma virus-transformed nonproducer cells. Such a result would also suggest that the murine type-C viral sequences have been deleted from each of the sarcoma virus genomes and that this is the explanation for the lack of sequences found in the RNA of such nonproducer cells. However, additional experiments will be necessary to prove that these viruses are in fact deletion mutants of the complete MuLV genome and to quantitate the portion of the genome deleted.

The current study seems to indicate a similarity in the genesis of both mammalian sarcoma viruses and avian sarcoma viruses. It has been demonstrated that several avian sarcoma viruses, in contrast to the murine sarcoma viruses, have the ability to both replicate and transform fibroblasts in cell culture. Analysis of the size of the sarcoma virus genome versus the helper genome has indicated that the competent sarcoma virus genome is larger in size than the helper virus genome (5, 6, 15). The larger sarcoma virus genome has been designated the a subunit, and the smaller helper virus genome has been designated the b subunit. A model has been suggested by Duesberg and Vogt (5, 6) that the b subunit has gained information, designated X, to form the larger a subunit. Thus a = b + X (15). Our own work indicates, in the case of two mammalian sarcoma viruses, that they

also have gained information not present in the initial helper virus genome. Although the size of the Kirsten and Harvey sarcoma genomes is smaller than that of the initial helper Kirsten or Moloney murine type-C viral genomes (18), by hybridization experiments our data demonstrate the gain of information (analogous to X) not present in the initial Kirsten type-C or Moloney type-C viruses. Thus, the difference between the competent avian sarcoma virus and the defective mammalian sarcoma virus might simply be the deletion of some of helper type-C viral information during or after the process of formation of the sarcoma virus. This deletion would render the sarcoma virus defective for replication but not for transformation. For both the avian sarcoma virus and mammalian sarcoma virus, however, a gain of information by initial helper virus genome would have occurred to produce the transforming virus. However, in neither the avian system nor as previously noted in the case of Ki-SV (24) can we yet identify the exact source of the information in either sarcoma virus which codes for the maintenance of transformation. Nevertheless, the formation of transforming type-C virus from the helper virus in both avian and mammalian systems is analogous to the process of transduction of information by bacterial viruses. In fact, sarcoma viruses defective for replication which are apparent deletion mutants derived from competent sarcoma viruses for replication have been reported for avian sarcoma virus as well (12). Whether or not such a process of recombination or transduction, or both, is necessary for the formation of other type-C viruses which can transform other types of cells, such as lymphoreticular cells, is unknown at this time. However, it is tempting to speculate that there are nononcogenic type-C viruses which acquire their oncogenicity through similar recombinational events (26) in the case of production of leukemia-producing type-C viruses as well as sarcoma-producing type-C viruses.

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